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FAX Message to: Judy Graham and David Kortum

From: Kathryn Mahaffey

The summary of the meeting "Health Effects and Exposure Symposium: Manganese" transmitted as part of this FAX has been cleared by Dr. Kenneth Olden, Director, NIEHS and Dr. James R. Fouts, Science Advisor to the Director, for publication in Environmental Health Perspectives. Recommendations on needed health effects research are presented on pages 2 and 3. In view of your questions on the NIEHS perspective on this topic, I draw your attention to these recommendations (in particular the second recommendation presented on page 2).

If you need additional information on this topic or clarification, please contact me. During the week of December 8 to 13, I will be in Research Triangle Park conducting the "Lead in the Adult" meeting (during the first three days of the week) and at NIEHS on Thursday and Friday. I can be reached through 919/541-1817 (Mrs. Karen Cowardin) or 919/541-3506 (Dr. Fouts).

# HEALTH EFFECTS AND EXPOSURE SYMPOSIUM: MANGANESE

March 12, 1991

Sponsored by the

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

and the

ENVIRONMENTAL PROTECTION AGENCY

Summary by

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* This summary is based on written abstracts and oral presentations at the March 12, 1991, meeting. The helpful comments of Dr. J. Routt Reigart, Dr. James R. Fouts, Dr. Fred Hochberg and Mrs. Betty Mushak are acknowledged.

## Recommendations

- Previous semi-quantitative estimates of the magnitude of manganese exposure that produce nervous system damage were derived from occupational exposures. Experience with other metals indicates that the biological availability of metals may differ widely and is influenced by valence state, size of particulate matter, complexation with other materials, and route of absorption. The bioavailability of manganese from MMT or products of MMT-combustion need to be compared with manganese from mines or smelters.

- Our overall understanding of the toxic effects of manganese, other than as observed at very high industrial exposures, is very limited at this time. Any prediction of outcome of low and moderate exposures to manganese would be extremely hazardous in light of the paucity of current information. Likewise, any action that resulted in a marked increase in population exposure would have very unpredictable results.

- Pharmacokinetic studies of manganese absorption, retention and tissue distribution after exposure to products of MMT combustion should be conducted. Exposures should include the inhalation and ingestion routes; exposure regimens should include chronic as well as short-term exposures at more than one dose. This work will help determine whether or not there are unusual features that distinguish between Mn derived from MMT combustion and Mn from other (non- MMT derived) occupational and nonoccupational manganese exposure and metabolism.

- Whether or not exposure to moderate quantities of manganese results in adverse effects on human health continues to be studied. Although extrapolation of data from animal species (including nonhuman primates) can provide valuable information on whether or not specific adverse health effects will be produced, human information is required for quantitative assessment of dose-response, especially at lower dose levels.

- Epidemiological studies of populations with long-term environmental exposure to manganese are needed to assess human dose-response. Manganese exposure produces adverse effects on several organ systems; however, neuro- behavioral and reproductive/early postnatal effects appear to be of particular concern. Several methodological problems exist in conducting useful epidemiological studies: identifying suitable biological indicators of manganese exposure and selecting sensitive and specific psychological and/or neurophysiological parameters to assess nervous system toxicity (especially in the newborn or young infant).

- Physiologically relevant markers of body burden of manganese may exist, but, chemical contamination can make these analyses appear poor predictors of body burden. Contamination of biological samples with manganese from products used to collect blood (e.g., manganese in stainless steel needles, manganese

contamination of anticoagulants) may obscure any association of body burden of manganese and environmental exposure. Other parameters, such as hair concentration of manganese, vary with hair pigmentation, further complicating study design. Relevant markers of manganese exposure, especially markers of cumulative exposure need to be established and validated.

- Additional analytical studies are needed to evaluate contamination of biological samples by manganese. Contamination can occur during sampling, separation, transport and chemical analysis. Reference laboratories need to establish procedures that improve detection of manganese contamination, including the use of standard reference materials and the technique of standard additions or "spiked" samples.

- Neurological signs and symptoms of full-blown manganism are quite characteristic of the disorder. However, indices of subtle, neuro-behavioral deficit (as produced by lower levels of manganese exposure) are much less well defined. Selection of tests of neuro-behavioral or neuro-motor function must be chosen to reflect the character of the neurological deficit that occurs at lower and moderate manganese concentrations.

- These studies should include both females and males. The age range of subjects evaluated should be as wide as possible. Methods used in such epidemiological studies should characterize the nature and duration of manganese exposures. Other factors that clearly modify the relationship between the dose of environmental manganese and the toxic effects, such as iron deficiency, must be measured as a source of variability.

## Introduction and Background

In May of 1990 the US Environmental Protection Agency (EPA) received a waiver application to introduce methylcyclopentadienyl manganese tricarbonyl (MMT) into unleaded gasoline. Part of the evaluation of this petition for waiver was an assessment of the potential health risks associated with emissions resulting from the proposed MMT use. The primary health-related issue was perceived to be potential effects associated with inhalation of combustion emissions of manganese tetroxide. Because of perceived gaps in knowledge of the effects of MMT and manganese, a symposium was organized to identify:

- current, ongoing studies of the health effects of manganese, particularly those that have not yet been published in the peer-reviewed, scientific literature; and

- research needs that would better define the nature of human dose and response to manganese-induced adverse effects on health.

This health effects and exposure symposium (March 12) was followed by a Risk Assessment Research Needs Workshop on March 13-15 that more specifically addressed research needed to improve the EPA's Office of Research and Development's preliminary risk assessment of manganese and MMT. The deliberations of those risk assessment issues have been summarized separately by the ORD of US EPA. The following summary of the March 12, 1991 symposium (held at NIEHS) is intended to reflect only the discussions of that one day symposium and does not necessarily focus on the same regulatory-related research issues. Rather, the Health Effects and Exposure Symposium held at NIEHS is more general in scope and reflects unresolved questions on manganese toxicity that could be the subject of general biomedical research.

Manganese excess has been known to produce a characteristic neurological syndrome, adversely affect reproduction, and alter respiratory function. The biochemical bases of these disorders are not clearly elucidated, but appear to involve possibly the strong oxidation-reduction potential of manganese (Donaldson et al., 1991). The manganese concentration of tissues is not a particularly useful guide to the production of signs and symptoms of manganese toxicity. For example, there is not a clear association between manganese tissue concentration and manganese-induced changes in the basal ganglia of the brain.

## Recent Epidemiology Studies on Health Effects of Manganese Exposures

Prolonged exposure to manganese particulate or fumes produces a stereotyped disorder referred to as manganism. Descriptions of human cases have come from Japan, Chile, Italy, Morocco, Mexico, Russia, and Rumania (Hochberg, 1991). The reported signs and symptoms are very similar among workers in various occupations (miners, ore-processors, welder, battery or electrode manufacturers) irrespective of the route of exposure. These routes have included inhalation of manganese particles, aerosols or fumes, or ingestion from food or water. Clinical manganism occurs in three discernable stages (Hochberg, 1991):

- Early behavioral abnormalities (referred to as "manganese madness" or Locura Manganica) which are characterized by acts that are compulsive and inappropriate for the situation. This phase may be preceded by anorexia and sleep disorders. There are changes in sexual activity and episodes of emotional instability. This phase follows manganese exposures of one to 10 years duration. It typically lasts for several weeks and is reversible depending on treatment and/or removal from exposure. If neither occur, the disease typically progresses to an "intermediate" phase after an interval of 2 to 6 months.

- The intermediate phase is characterized by muscle pains in the back and extremities and neurologic symptoms including incoordination of arm, hand, leg and gait, bradykinesia, masked facies and hypophonia (Hochberg, 1991).

- In advanced manganism, neurologic deficits include tremor, dystonic rigidity, gait dystonia and rarely disordered sensation. Alterations are seen in the basal ganglia and corticospinal tract. The abnormal gait is characterized by toe walking. The tremor of manganism is both a resting and an action tremor of wide amplitude. It is of a different character than that observed in Idiopathic Parkinson's Disease. The movement disorder in manganism is a disorder of wide amplitude involving the proximal arms and, rarely, the whole body. The tremor of Idiopathic Parkinson's Disease is of small amplitude, involving the fingers or wrists.

Although very high doses of manganese produce the neurologic disorder described above, relatively little is known about the clinical outcome of moderate to low manganese exposures. Because muscle incoordination and movement disorder are characteristic of manganism, on-going studies of the effects of manganese exposure in steel smelters have relied on neurobehavioral and electrophysiological assessments. Examples of tests in psychological batteries for manganese effect include: finger tapping, simple and complex reaction time, and finger dexterity. Alteration in performance on these tests, associated with increased manganese exposure, have been interpreted as a pre-clinical stage of full-blown manganism related to long-term exposure to high manganese levels (Iregren and Wennberg, 1991).

Another cross-sectional study was carried out to assess if an association exists between manganese exposure and subtle changes in psychomotor and cognitive functions. Subjects of this study either worked at an open-cut manganese mine on Groote Eylandt in northern Australia or were iron miners on a separate island that had much lower environmental manganese exposures (Hart et al., 1991). Neuropsychological tests included an assessment of general intelligence, a profile of mood states, paired-associated learning, critical flicker fusion, auditory/verbal learning, hand/eye co-ordination, reaction time and tremorometry. These tests revealed no differences between the group living in the higher manganese region and the group of iron miners. This finding is difficult to interpret because the biological measures of exposure (i.e., internal dose) did not identify an association with the quantity of manganese in the environment.

Some of the inhabitants of this high manganese region of Australia have substantial neurological symptoms. Evaluation of a subgroup of the afflicted individuals indicated that there is no support for an association of manganese exposure with this neurological disorder. This disorder which is a well-recognized degenerative disorder (olivopontocerebellar degeneration) was inherited as an autosomal dominant genetic disorder. A second disorder recognized in this population is Type I Ehler-Danlos syndrome, which is also inherited as an autosomal dominant disorder and therefore could not, by definition, be associated with manganese exposure (Hochberg, 1991).

Behavioral abnormalities characterize the early stages of manganism. In addition, hair manganese concentrations have been found to be elevated among prisoners with a history of particularly violent behavior (Gottschalk, 1991). This difference in hair-manganese

concentration is present at the time of incarceration of the subjects and so does not reflect in-prison exposures. The subjects were grouped by ethnicity because of the known association of hair pigmentation with hair manganese content. A mechanistic explanation for these observations is not obvious.

One problem present in cross-sectional studies that evaluate the prevalence of signs and symptoms of manganese exposure, is the difficulty of estimating past exposure. Typically, estimates of ambient manganese exposure are derived from whatever industrial hygiene measurements may be available. However, these do not fully reflect manganese intake and do not identify individual characteristics that determine internal dose of manganese. A common problem in all studies of manganese exposure or manganese-induced health effects is the absence of a usable biomarker of body stores of manganese. Problems of contamination of samples with manganese greatly limit the utility of most analyses of blood or urine samples. For example, most syringes used to obtain blood samples use a stainless steel needle that may contaminate the blood flowing through it with manganese. Hair manganese has been used as a biological marker for manganese. The limitations of hair as a kind of biological marker are recognized. For example, it is known that the degree of pigmentation of hair is directly associated with the concentrations of manganese in hair. This should greatly influence selection of appropriate control subjects.

Biokinetics and Susceptibility to Manganese

The biokinetics of manganese (e.g., the patterns of absorption, retention, and deposition of manganese into various tissues) are moderately well understood. Once absorbed across biological membranes (i.e., the gastrointestinal tract, the lung alveoli) manganese is transported on serum proteins (especially transferrin) and accumulates in organs including the kidney, liver, and brain. Inhaled manganese is retained, with the lung serving as an internal depot that slowly releases manganese to other tissues such as the brain (Oberdorster and Wieczorek, 1991). Lung retention of inhaled manganese is dependent on the deposited dose. Rapid clearance rates after high exposure to $^{54}MnCl_2$ aerosols are about 10 times greater than after low exposures (Oberdorster and Wieczorek, 1991). Manganese can be released from tissue and is excreted via the bile and urine. Fecal manganese is the main excretory route for ingested and inhaled manganese. Manganese uptake into the brain and clearance from the brain occur at a low rate; these are not proportional to blood manganese concentration. When animals have been exposed to high concentrations of manganese, homeostatic mechanisms appear to accelerate manganese elimination from liver and kidney (Oberdorster and Wieczorek, 1991). Research questions include what factors in addition to dose affect tissue deposition and release of manganese, e.g., chemical species.

Whether or not MMT or manganese oxides resulting from combustion of MMT have different patterns of tissue deposition compared with other manganese species is unclear. The valence state of manganese achieved at the time of exposure may be a function of the type of industrial process and the temperature of combustion. Answers to this question will require additional research that investigates tissue deposition of manganese in emissions

from the gasoline engine. This information will provide data that helps establish whether or not findings from inorganic manganese can be generalized to MMT and MMT emission products.

Not everyone is equally susceptible to the toxic effects of manganese. Although most epidemiology studies have been carried out on adult males with occupational manganese exposures, this group is not the most vulnerable to manganese effects. Infants and people with high absorption of iron, absorb and retain a greater fraction of the manganese that they ingest (Lonnerdal, 1991). For infants, the practice of using high concentrations of manganese in formulas has been phased out because of concern over increasing tissue manganese burden. Studies in monkeys and rats have shown that when iron absorption is high, manganese absorption is also increased. Adult males are not typically iron deficient unless the diet is unusually limited in quality. Pathological conditions in which adult males over-absorb iron (e.g., hemochromatosis) could result in increased tissue deposition of manganese, as well as iron.

Under physiologically stable conditions the concentration of manganese in the brain is kept low by tight homeostatic control of absorption and excretion and by the blood brain barrier. At the cellular level, the powerful redox properties of manganese underscore its potential effects on metal-activations and metalloenzymes. $Mn^{2+}$ binds to ligands also preferred by $Mg^{2+}$ or $Ca^{2+}$. Manganese competes with $Ca^{2+}$ for transport across cell and mitochondrial membranes. Because of the physiological importance of magnesium and calcium, competition by changes in the concentrations of $Mn^{2+}$ could be physiologically adverse. Transport of manganese across the blood-brain barrier utilizes the carrier protein transferrin, a protein that typically carries iron. In experiments with rats, high iron concentrations depressed manganese uptake into the brain (Ashner, 1991). Brain manganese uptake was increased when more manganese was bound to transferrin. The overall patterns of deposition of iron and manganese in neural tissue suggests these cations may share a number of common transport pathways. At the mitochondrial level the extremely slow kinetics of $Mn^{2+}$ efflux from mitochondria may be expected to contribute both to $Mn^{2+}$ accumulation in brain mitochondria and to its slow clearance from brain tissue (Galvin, 1991).

## Neurotoxicity and Developmental Effects of Manganese Excess

Neurotoxic effects of manganese have been examined in primate models. Following manganese exposure by inhalation, manganese is slowly released and redistributed from site of initial deposition. Kinetic studies on the distribution of radioactivity (Mn 54) in the body regions of primates showed that manganese was slowly released from the chest (i.e., lung) to the head (i.e., central nervous system). Over a cumulative dose range (Newland, 1991) between 200 and 2000 mg/kg in primates, one site of CNS localization of manganese is in the globus pallidus. This is prior to the onset of signs and symptoms of

manganism. Neuromuscular impairment appears to be an early indicator of manganese-induced dysfunction in this primate model. Another early indicator of manganese effects to be delays in response times for conditioned responses.

Difficulties that arise in establishing a dose-effect or dose-response model for manganism are complicated by the biokinetics of manganese. Patterns of tissue distribution and
redistribution of manganese over time complicate association between long-term neurotoxic effects and tissue manganese concentrations. Current interpretations of these findings indicate the tissue manganese concentrations at the time of development of clinical disease do not reflect what tissue manganese concentrations were at the time the disease process was initiated.

Whether or not the aging process would contribute to expression of chemically induced neurotoxic disease in a population previously compromised by exposure to neurotoxic chemicals, remains predominantly theoretical (Weiss, 1991). Examples exist in which rats and primates exposed to methyl mercury early in life, but not throughout their life-times, develop neurological indicators of methyl-mercury poisoning in mid-to-late life. The aging process reduces the compensatory capacity of the nervous system and may uncover damage due to neurotoxic chemicals.

There is little to support manganese excess as teratogenic. Manganese will cross the placenta; however, only about 3 to 7% of a dose reaches the developing fetus (Kay, 1991).

A-91-46
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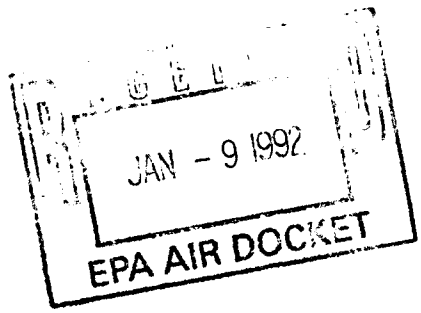
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460




OFFICE OF
AIR AND RADIATION

MEMORANDUM

SUBJECT: Telephone Conversation with Toyota Technical Center, U.S.A., Inc. Staff

FROM: David J. Kortum, Environmental Engineer
Fuels Section

David J Kortum 1/2/92

TO: Docket A-91-46 (LE-131)

The purpose of this memorandum is to describe a telephone conversation which I had with Bob Babcock of the Toyota Technical Center staff on December 16, 1991 regarding the application by the Ethyl Corporation for a fuel waiver for HiTEC 3000.

Mr. Babcock explained Toyota's reasoning behind the way in which testing of its Camry on MMT-containing fuel was conducted. He explained that Toyota believes that evidence has been presented in previous submissions which indicates that Toyota's "9-Laps" procedure results in catalyst degradation more representative of actual in-use driving. Mr. Babcock also stated that the use of a single vehicle as both a test and control vehicle is satisfactory as a test procedure because the clear fuel portion of the test took place after the portion of the test when MMT-containing fuel was used. Hence, he explained, Toyota believes that the MMT-effect for HC emissions would actually, if anything, be minimized by this approach.